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SYNCHRONIZATION OF RAPID GLOBIN EXPRESSION IN MURINE ERYTHRO-LEUKEMIC CELLS

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ABSTRACT

The addition of butyric acid (BA) to murine erythroleukemia cells (MELC) produces the expression of primarily A and E_2 hemoglobins while DMSO incubation produces the expression of primarily A hemoglobin. Preincubation of MELC with DMSO followed by BA induction accelerates the expression of hemoglobins in a synchronous manner, yielding the preferential synthesis of α and y globins, and E_2 hemoglobin.

INTRODUCTION

In mouse fetal development after day 8, embryonic nucleated erythrocytes, which are derived from the yolk sac blood islands, synthesize three types of embryonic hemoglobins designated E_I (x_2y_2), E_{II} (α_2y_2), and E_{III} (α_2z_2). After day 12, the fetal liver becomes active releasing non-nucleated erythrocytes into the circulation which produce primarily adult hemoglobins ($\alpha_2\ \beta_2$) [1].

Murine erythroleukemic cells (MELC clone T3CL2) can be induced to differentiate with DMSO or BA [1] and produce different types and quantities of hemoglobins with each inducer. Murine erythroleukemic cells (MELC clone T3CL2) can be induced to differentiate with DMSO to produce primarily A hemoglobin ($\alpha_2\ \beta_2$) while those induced with butyric acid (BA) produce A ($\alpha_2\ \beta_2$) and E_2 ($\alpha_2\ y_2$) hemoglobins [2]. The addition of hemin to MELC stimulates hemoglobin synthesis, heme biosynthesis and globin m-RNA transcription [3].

By using heterogeneous cell populations to analyze the cellular biochemistry, some problems are created. The data presented in this study suggests a rapid switch in hemoglobin gene expression can occur by incubating the cells initially with DMSO followed by butyric acid. The butyric acid induction yields a rapid expression of y globins which would facilitate subsequent biochemical experiments and provide a more synchronous model system to study gene switching.

MATERIALS AND METHODS

The Friend erythroleukemic cells T3CL2 were grown in α medium as previously described [4]. The cells were induced to differentiate by incubating them with DMSO (1.5%) for 1-3 days followed by butyric acid (1 mM) for 1-2 days. The cells were then labeled with H^3 leucine in a leucine free MEM medium. The hemoglobin obtained was separated by isoelectric focusing and the globins were separated by acid urea electrophoresis. In both procedures, the proteins were analyzed by fluorography as previously described [4].

RESULTS AND DISCUSSION

The addition of DMSO to T3CL2 cells produces primarily A hemoglobin. The level of synthesis can be increased if hemin is added for 4 hours or 24 hours. In contrast to DMSO, the addition of BA to T3CL2 cells produces A and E_2 hemoglobins. If the cells are initially incubated with DMSO and then switched to BA for 1 day, there is a rapid synthesis of E_2 hemoglobin contained in band 4 and band 1 (Fig. 1A). Normally the addition of BA for one day results in very low levels of globin and hemoglobin synthesis [2]. Thus by combining the two inducers, it appears that preincubation of the cells with DMSO facilitated the subsequent expression of hemoglobins induced with BA. The addition of hemin for 24 hours results in the increased synthesis of E_2 and A and a decreased synthesis of band 1 [4]. The hemin effect suggests that there is a heme deficient state present in DMSO or BA induced cells which can be eliminated by the addition of hemin. The data also suggests that in heme deficient conditions with BA induction, E_2 will be synthesized in preference to A (Fig. 1A).

In order to determine if the switching of inducers affected the globin synthesis, triton x-100 acid urea electrophoresis was used (Fig. 1B). The results demonstrated that the switching from DMSO to BA induction increased the synthesis of the α and y globins. The addition of hemin to DMSO or BA induction increased the synthesis of α and β globins.

In order to determine if there was a time factor necessary for DMSO incubation, cells were incubated with DMSO for 24 or 48 hours and then placed in BA for 24 hours. It was found that the quantity of hemoglobin synthesis was higher with the switching of the inducers than with either inducer alone (Fig. 2). This suggests that DMSO induction produced a series of events which facilitated the rapid expression of hemoglobins after BA was added.

It has been shown that a number of events occur in early differentiation prior to the expression of heme, globin, and hemoglobin. These include a decrease in cell volume, a transient G_1 block, specific ion movements and an increase in cyclic AMP. It is possible that in the differentiation of MELC, a series of discrete steps occurs prior to terminal differentiation. In our experiments, it appears that during the incubation of the cells with DMSO the early events necessary for differentiation occurred which primed the cells to be rapidly induced when BA was added. The result was a synchronous expression of hemoglobin after the addition of BA.

FIGURES

Fig. 1A

Fluorogram of isoelectric focusing of T3CL2 hemoglobins. The cells were incubated with DMSO for 4 days or with DMSO for 3 days and switched to butyric acid for a day. Hemin was added for 4 hours or 24 hours.
(a) DMSO (4 days), (b) DMSO (4 days) + hemin (4 hours), (c) DMSO (3 days) + hemin (1 day), (d) DMSO (3 days) + BA (1 day), (e) DMSO (3 days) + BA (1 day) + hemin 4 hours, (f) DMSO (3 days) + BA (1 days) + hemin (1 day).

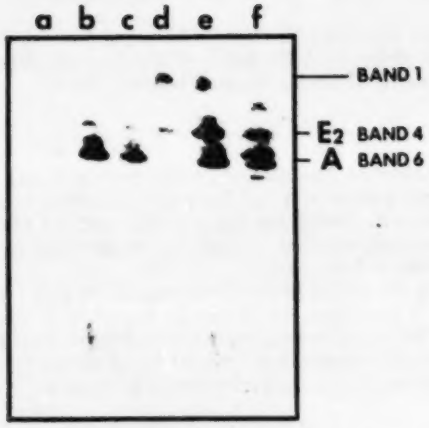

FIGURE 1A

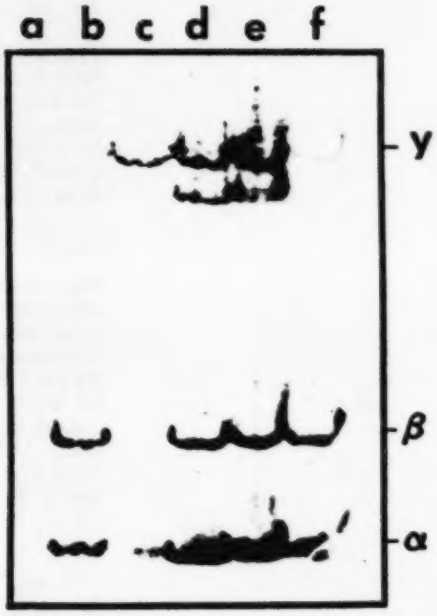

FIGURE 1B

FIGURE 2

Fig. 1B

Fluorogram of acid urea Triton x 100 electrophoretic separation of globins derived from T3CL2 cells. The cells were incubated with DMSO for 4 days or with DMSO for 3 days and then switched to BA for 1 day. Hemin was added for 4 hours or 24 hours.
(a) DMSO (4 days), (b) DMSO (4 days) + hemin (4 hours), (c) DMSO (3 days) BA (1 day), (d) DMSO (3 days) + BA (1 day) + hemin (1 day), (e) DMSO (3 days) + BA (1 day) + hemin (4 hours), (f) DMSO (3 days) hemin (1 day).

Fig. 2

Fluorogram of isoelectric focusing of T3CL2 hemoglobins. The cells were induced with DMSO and then switched to BA for 1 day. Control hemoglobins were obtained by incubating cells with DMSO for 2 and 3 days and BA for 2 and 3 days. With the culture conditions used, minimal hemoglobin is detected by incubating the cells in BA for 1 day and hemin for 4 hours.
(a) DMSO (2 days), (b) DMSO (2 days) + hemin (4 hours), (c) DMSO (1 day) then BA (1 day), (d) DMSO (1 day) then BA (1 days) + hemin (4 hours), (e) DMSO (3 days), (f) DMSO (3 days) + hemin (4 hours), (g) DMSO (2 days) then BA (1 day), (h) DMSO (2 days) then BA (1 day) + hemin (4 hours), (i) BA (2 days), (j) BA (2 days) + hemin (4 hours), (k) BA (3 days), (l) BA (3 days) + hemin (4 hours).

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